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BACTERIAL LEAF SCORCH OF AMENITY TREES:

A WIDE-SPREAD PROBLEM OF ECONOMIC SIGNIFICANCE TO THE URBAN FOREST

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September 11-12, 2001
Rutgers University
Cook College

ABOUT THE CONFERENCE

Bacterial leaf scorch (BLS) of amenity trees is caused by the bacterium *Xylella fastidiosa*, a xylem-limited pathogen that causes water stress resulting in leaf scorch, decline, and eventual death of affected trees. Recent surveys indicate that BLS is widespread throughout the eastern half of the United States. In New Jersey, BLS primarily affects red and pin oaks planted as landscape and street trees. Until recently, the disease was limited to southern and central regions of New Jersey, but is now found in communities throughout the State. We estimate that as many as 30% of oaks are affected by this disease in some municipalities. Not only is BLS considered a threat to northern red oak, the state tree of New Jersey, but it also has potential to devastate other species of oak such as pin and scarlet, as well.

In spite of its widespread distribution, little is known of the biology of BLS, how it spreads, source(s) of bacterial inoculum, and methods for early disease detection. Without this basic information, development of effective management strategies is difficult. With no current, cost-effective rescue technology, arborists and land managers are forced to remove trees in established communities, residential parks, and golf courses to prevent substantial liabilities that may result from falling branches in declining trees. Moreover, tree removal is expensive, and replacement trees take years to become attractive and valuable additions to affected landscapes. If uninvestigated and untreated, BLS will continue to debilitate municipalities where widespread overplanting of red and pin oaks has occurred.

This symposium was sponsored by the USDA Forest Service, Rutgers University, the New Jersey Department of Environmental Protection Division of Parks and Forestry, and the New Jersey Community Forestry Council. From this symposium, we have compiled a position paper on BLS that represents a synthesis of conference proceedings and published literature. In it, we focus on disease etiology, development and spread, relationships between host plant and disease vectors, detection, and management. Furthermore, a treatment of avenues for further study is presented. The best approach to possible management of BLS will most likely entail the coordinated efforts of university, state, and federal forest and agriculture agencies, urban managers, and community groups. Common goals may be realized through a team approach to better understand and manage the disease.

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INTRODUCTION

Xylella fastidiosa is a plant pathogen of major economic and ecological importance in both agriculture and in the urban forest. The xylem inhabiting bacterium has an extensive host plant range and is transmitted from plant to plant by many species of xylem feeding homopteran insects. At least 15 different diseases of economic importance are caused by *X. fastidiosa*, many of which exhibit leaf scorch as a symptom. Amenity trees affected by *X. fastidiosa* have been reported from states east of the Mississippi River and in Texas. In many such trees, scorch symptoms appear in late summer and are often mistaken by the lay public as early senescence or drought stress. Infection in amenity trees is chronic, taking some infected trees years to die depending on host species and environmental conditions.

Symptoms associated with *X. fastidiosa* on amenity trees include an irregular, marginal necrosis often accompanied by a red or yellow halo. These symptoms usually occur in mid- to late-summer on leaves of one or more branches in the canopy. As the infection progresses over several years, branches die and the tree declines to the point where it dies, must otherwise be removed, or succumbs to another disease or insect pest. The process of tree decline may occur quickly or slowly depending on the tree and the environment. Epicormic branching is often evident on severely diseased trees, and scale insects, borers, Armillaria root rot, and other biotic diseases may express themselves as secondary pests.

In the northeastern United States, detection of BLS in amenity trees has increased. In New Jersey, for example, the disease was first identified in the mid-1980s in several southwestern counties that border the Delaware River. A recent survey of the disease indicated that BLS has since been detected in more northern and eastern counties and is now found in communities in most areas of the State. In some residential neighborhoods, up to 30% of mature oaks planted as street trees are affected by the disease.

BACTERIAL LEAF SCORCH

Bacterial leaf scorch (BLS) of amenity trees is a bacterial infection of shade trees species such as oak, sycamore, elm, mulberry, and maple throughout the eastern United States (Table 1). BLS is one of a group of diseases caused by a xylem-limited bacterium, *X. fastidiosa*, which has major economic and ecological importance in both agriculture and in the urban forest. *X. fastidiosa* is distributed throughout the Western Hemisphere, has a very wide host range, and causes diseases in a number of economically important hosts including peach, grape, plum, pear, periwinkle, alfalfa, almond, citrus, oleander, and coffee. The bacterium also resides in alternative hosts, many of them common landscape ornamentals and weeds, where it may cause no discernible symptoms of disease. *X. fastidiosa* is transmitted from plant to plant by many species of xylem-feeding homopteran insects. In many hosts, the identity of specific insects that vector the pathogen is unknown.

Table 1. Representative shade tree hosts affected by BLS [44; 62]

Acer rubrum	Red maple
A. negundo	Boxelder
A. saccharum	Sugar maple
Cornus florida	Flowering dogwood
Liquidambar stryraciflua	Sweet gum
Morus alba	White mulberry
Ulmus americana	American elm
Platanus occidentalis	American sycamore
P. x *acerifolia*	London plane
Quercus sp.	Oak

HISTORY

The existence of the pathogen ultimately deemed responsible for BLS and the other economically important diseases has been suspected since the late 1800s (Table 2). In 1892, Newton B. Pierce, state plant pathologist of California, first studied a disease resulting in scorch and decline of grapevine (*Vitis vinifera* L.) known at the time as California vine disease. Although the disease was eventually named for him, Pierce himself could not isolate, culture, nor identify the causal agent, suspecting that a "minute microorganism" was involved [59].

Table 2. Milestones in the study of *Xylella fastidiosa*

First report of Pierce's disease and phony peach disease	1890s
Graft transmission of Pierce's disease and phony peach disease	1939
Leafhopper transmission of Pierce's disease and alfalfa dwarf	1946
Tetracycline suppression of Pierce's disease symptoms	1971
Association of rickettsia like bacteria with Pierce's disease and phony peach disease	1973
Isolation of bacterium from infected grapevine	1978
Pierce's disease bacterium associated with leaf scorch	1980
Description of the *Xylella fastidiosa* species	1987

In the late 1930s and early 1940s, Hewitt [29; 30] reported that Pierce's disease of grapevine, now thought to be caused by a virus, was transmissible by grafting and by insect vectors. Suppression of disease symptoms by antibiotics led Hopkins and Mortensen [34] to conclude that Pierce's disease might be caused by a mycoplasma. In 1973, however, Goheen and co-workers [24] using electron microscopy detected an organism in the xylem vessels of infected grapevine leaves. Because of its rippled cell wall and fastidious nutrient requirement, the causal agent was reclassified as a rickettsia-like bacterium. Study of this organism was significantly advanced in 1978 when a culture medium sufficient to isolate and grow the bacterium in vitro was developed [17]. It was not until 1987, however, that this organism was properly described as a bacterium closely related to the bacterial plant pathogen *Xanthomonas*, and was given the name *X. fastidiosa* [70].

X. fastidiosa is now known to be a xylem-inhabiting bacterium that causes leaf scorch and decline of many economically important hosts (Table 3) and alternative, or natural, hosts (Table 4). The bacterium was first observed in association with shade tree leaf scorches in 1980 [28], and since, pathogenicity to oak, elm, sycamore [28], mulberry [40], and maple [64] has been demonstrated.

Table 3. Some of the economically important diseases caused by *Xylella fastidiosa*, some of which have host specific strains and may not be cross pathogenic [62]

Alfalfa dwarf	Oleander leaf scorch
Almond leaf scorch	Phony peach disease
Citrus variegated chlorosis	Pear leaf scorch
Coffee leaf scorch	Periwinkle wilt
Elm leaf scorch	Pierce's disease of grapevine
Maple leaf scorch	Plum leaf scald
Mulberry leaf scorch	Sycamore leaf scorch
Oak leaf scorch	

THE PATHOGEN

X. fastidiosa is a Gram-negative, aerobic rod that is nonmotile and often possesses a rippled cell wall that may or may not have microfibrils. The original type description of *X fastidiosa* included 25 bacterial strains recovered from 10 different hosts as a single species [70]. However, many differences between strains in characteristics such as host range, pathogenicity, immunogenicity, nutritional requirements, genetic homology, and cross pathogenicity have since been reported, indicating possible reclassification of this organism to the pathovar or subspecies level [56]. The entire genome of a strain of *X. fastidiosa* isolated from citrus has been recently sequenced (the first plant pathogen for which this has been performed) [65]. Based on what is known in other microbes, the *X. fastidiosa* genome encodes for proteins involved in cell-cell interaction, degradation of plant cell walls, synthesis of toxins, and pathogenicity [41].

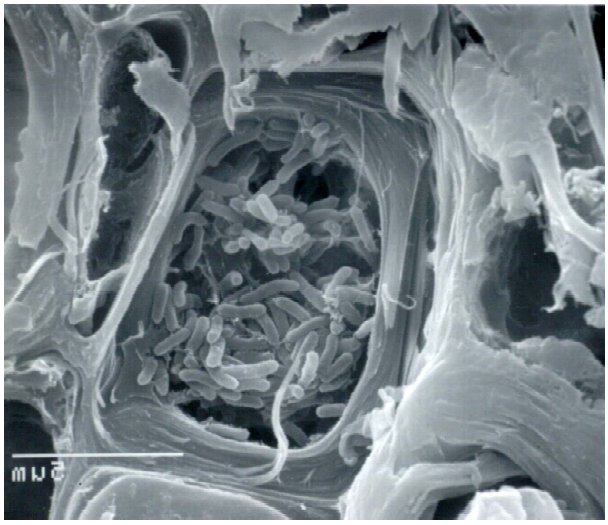

Figure 1. Xylem cell occluded with bacterial cells. (Photograph courtesy of R. Jordan.)

DISEASE DEVELOPMENT

In trees affected by BLS, scorch symptoms appear in late summer and are often mistaken by the lay public as early senescence or drought stress. Chronic symptoms in host plants result because *X. fastidiosa* is specifically located in the tracheary elements, tracheids, vessels, and intercellular spaces of xylem tissue [36; 47] (Figure 1). In leaf scorch diseases such as BLS of elm, bacterial populations are great in the primary and secondary veins of symptomatic leaves [28] and fluctuate seasonally [66]. Compared to other plant tissues, xylem fluid is nutritionally poor but does consist of amino acids, organic acids, and inorganic acids. Concentrations of the amide amino acids (glutamine and asparagine) are high and these compounds are utilized by both the bacterium [16] and its insect vector [6; 7; 26]. Xylem fluid composition and quality varies between hosts and within a host, and varies seasonally, diurnally, and with the health and age of the host [4; 5; 8; 26]. This variation may have a profound impact on disease development and vector behavior.

Symptom development is a function of the rate and extent of bacterial colonization as well as the intensity of the host plant response. High titers of bacterial cells in the lumen of tracheary elements, overproduction of pectins produced by the host in response to infection, and eventual formation of embolisms contribute to a plugging of xylem vessels which is thought to contribute to low level water stress in affected tissues [20; 47; 56]. When prolonged, this low level water stress, which is accompanied by reduced xylem function, changes in water relations, reduced starch reserves, and reduced tissue available for photosynthesis, results in scorching and accelerated leaf senescence [25; 39].

Other products of host origin, such as gums and tyloses, may be more important in the successful defense response of tolerant hosts to bacterial infection where total restriction of the vascular pathogen is necessary for resistance. In such cases, infected plants may survive to produce new growth to compensate for blocked, nonfunctional xylem tissue [23].

PRIMARY AND ALTERNATIVE HOSTS

Alternative hosts (Table 4) are considered those plants that support the growth of *X. fastidiosa*, that may or may not be symptomatic, and that may serve as a source of inoculum for the more economically important primary hosts. The role played by alternative plant hosts in the ecological relationships between the disease, insect vectors, and primary plant hosts is not clear. In fact, from an insect point of view, what

we call an alternative host could actually be a primary feeding or reproductive host for the insect. Andrew McElrone of the University of Maryland has shown conclusively that Virginia creeper is a host, and the pathosystem for *X. fastidiosa* is dramatically affected by drought in this plant.

Table 4. Some alternative hosts of *Xylella fastidiosa*

Aesculus x *hybrid*	Yellow buckeye [44]
Ampelopsis arborea	Peppervine [35]
Artemisia vulgaris	California mugwort [22]
Baccharis halimifolia	Eastern baccharis [35]
Callicarpa americana	American beautyberry [35]
Celastrus orbiculata	Oriental bittersweet [44]
Cornus florida	Flowering dogwood [44]
Cynodon dactylon	Bermuda grass [22]
Fragaria sp.	Wild strawberry [60]
Hedera helix	English ivy [44]
Montia linearis	Miner's lettuce [60]
Parthenocissus quinquefolia	Virginia creeper [35]
Parthenocissus tricuspidata	Boston ivy [22]
Paspalum dilatatum	Dallis grass [22]
Rhus sp.	Sumac [35]
Rubus procerus	Blackberry [35; 60]
Sambucus canadensis	American elder [35]
Solidago fistulosa	Goldenrod [35]
Sorghum halapense	Johnson grass [69]
Trifolium repens	Ladino clover [22]
Vinca minor	Periwinkle [60]
Vitis sp.	Wild grape [22; 44]

BLS OF SHADE TREE SPECIES

Several diseases caused by BLS, elm leaf scorch, sycamore leaf scorch, and oak leaf scorch, have been well characterized and have great economic impact from an aesthetic or commercial point of view.

Elm

Of the 3,000 elms within the monumental core of Washington, D.C., Jim Sherald of the National Parks Service reports that 30% of them are infected with BLS. Symptoms of BLS of elm include an irregular, necrotic pattern along the leaf edge that is often preceded by a chlorotic halo. The irregular scorching, which is quite different from the uniform pattern caused by drought, progresses from the margin of the leaf tip to the mid-vein. Symptoms progress from older to younger leaves; indeed, some leaves at the tip of the branch may not exhibit symptoms. American elms affected by BLS are highly susceptible to Dutch elm disease, which is the usual reason such trees eventually die and are removed.

Sycamore and London Plane

Jim Sherald has observed that about 80% of a 130-tree planting of sycamore in East Potomac Park is affected by BLS. In Washington, D.C., BLS in sycamore is a chronic disease, and it may take a considerable period before affected trees die. Symptoms appear late in the season as an interveinal leaf scorch with a small chlorotic halo. As in elm, the disease progresses from older to younger leaves. Recently, trees lining the walkway up to the west front of the Capitol building were sycamores; because of

BLS, many of them are now reduced in size, and a lot of dead wood has been removed. Sycamore seedlings that are inoculated with *X. fastidiosa* are reduced in growth and exhibit dieback characteristic of BLS.

Bartlett Tree Experts, a commercial tree care company, cares for properties that have trees with BLS. The Bartlett Tree Research Laboratories support the company's commercial operations with diagnostic services. This facility has confirmed the disease, most commonly for trees in the red oak group, from central New Jersey as far south as Tallahassee, Florida, the mid-western states, and in Texas. Bruce Fraedrich of this research facility has been monitoring a planting of 2,000 Bloodgood London plane on 15-foot spacing in Charlotte, North Carolina. The first tree died from BLS within 7 years after planting, and now incidence of the disease is 75%.

Kerry Britton of the USDA Forest Service has studied the incidence of BLS in street trees in Athens, Georgia, and estimates that up to 50% of these trees are affected by BLS. She suspects that BLS has been present in sycamore silage plantations and natural stands as part of a disease complex with the dieback fungi *Ceratocystis fimbriata* and *Botryosphaeria rhodina* since the 1950s but, until recently, its role remained unidentified. "Sycamore decline," as it was once called, varies geographically. Trees of southern provenances grown north of the origin of the seed source grow more quickly and are more resistant to decline than seed sources of more northern origin. Britton speculates that disease susceptibility is related to xylem vessel morphology or dormancy timing. Britton has also shown that isolation frequency of *X. fastidiosa* from infected trees goes down in the winter months, indicating that populations of bacteria diminish in winter.

Oak

Figure 2. Symptoms of marginal leaf scorch on northern red oak. (Photograph courtesy of A. B. Gould.)

In the Constitution Gardens near the Vietnam War Memorial, Jim Sherald reports that 20% of oaks are affected by BLS. In the early stages of this disease, portions of the tree remain unaffected, while other branches exhibit symptoms typical of the disease. Infected leaves in red oaks exhibit a pronounced, marginal discoloration with a dull red or yellow halo between scorched and green tissues (Figure 2). Due to determinant growth, all leaves on oak develop symptoms at the same time. As the disease progresses, more branches develop symptoms. Within plantings, disease incidence usually appears randomly; trees neighboring severely affected trees are often not affected. Leaf symptoms in pin oak are not as distinct, but the distribution of the disease within the canopy and between trees is the same.

In all known oak hosts, symptoms usually occur in mid- to late-summer on leaves of one or more branches in the canopy. Affected leaves may curl and drop prematurely. As the infection progresses over several years, branches die, and the tree declines. Affected trees eventually decline to the point where they must be removed [28]. The process of tree decline may occur quickly or slowly depending on the tree or the environment. Epicormic sprouts can be prominent on severely diseased trees [15], and scale insects, borers, Armillaria root rot, and other biotic diseases may be present as secondary pests [61].

As can be seen from Table 5, a number of oak species from the southern states to New England serve as hosts for *X. fastidiosa*. In Florida, Ed Barnard of the Florida Division of Plant Industry has documented the disease in populations of turkey oak throughout the State. BLS is present on turkey oak from the western regions of the Florida panhandle to the southern tip of the State. As is typical with this disease in other oaks, symptomatic and asymptomatic turkey oaks are dispersed within the same population of trees. Leaf scorch in turkey oak is not well defined; however, and Barnard observed that the internodal length of infected trees is shorter than in asymptomatic trees. Barnard speculates that *X. fastidiosa* may be associated with a disease syndrome known as oak decline that occurs periodically in Florida. Other pathogens associated with this decline include *Armillaria tibescens* (shoestring root rot) and *Ganoderma lucidum* (a white-rot conk fungus).

Table 5. Oaks known to be affected by bacterial leaf scorch [62]

Host	Location
Black oak (*Quercus velutina*)	New Jersey[Z]
Bluejack oak (*Q. incana*)	Florida [9]
Bur oak (*Q. macrocarpa*)	Kentucky [27]
Chestnut oak (*Q. prinus*)	Tennessee, New Jersey[Z] [42]
Laurel oak (*Q. laurifolia*)	Florida [9; 35]
Live oak (*Q. virginiana*)	Florida [9; 49]
Northern red oak (*Q. rubra*)	Kentucky, Tennessee, mid-Atlantic, and northeastern states [27; 28; 42]
Pin oak (*Q. palustris*)	Kentucky, Tennessee, and northeastern states [27; 42]
Post oak (*Q. stellata*)	Florida, Tennessee, New Jersey[Z] [9; 42]
Scarlet oak (*Q. coccinea*)	Tennessee, northeastern and mid-Atlantic states [28; 42]
Shingle oak (*Q. imbricaria*)	Kentucky [27]
Shumard oak (*Q. shumardii*)	Florida, New Jersey [49; 50]
Southern red oak (*Q. falcata*)	Florida [9; 35]
Swamp white oak (*Q. bicolor*)	Tennessee, New Jersey[Z] [42]
Turkey oak (*Q. laevis*)	Florida [9]
Water oak (*Q. nigra*)	Florida, Tennessee [35; 42]
White oak (*Q. alba*)	Tennessee [42]
Willow (*Q. phellos*)	Tennessee [42]

[Z] Buckley, R. J., Rutgers Plant Diagnostic Laboratory (personal communication).

In New Jersey, BLS causes leaf scorch and decline on primarily mature red and pin oaks planted as landscape and street trees; incidence on other oaks and shade tree species is rare. The disease was first identified in Camden, Gloucester, and Burlington Counties in the mid 1980s and has since been detected in more northern and eastern counties of the State (Figure 3). In some communities, municipal arborists estimate that the disease affects up to 30% of oaks in some residential neighborhoods, parks, cemeteries, and golf courses.

To assess the spread and impact of BLS on New Jersey's oak tree population, a statewide survey, funded by a $95,000 state appropriation (P.L. 2001, c. 8), was conducted in 2001 by the New Jersey Forest Service-Community Forestry Program with planning and educational assistance by Rutgers University [50]. Of the 1372 oaks sampled in this program, 39% were confirmed as positive for BLS by the Rutgers Plant Diagnostic Laboratory. Of these oaks, over 90% were red (the state tree of New Jersey) or pin (one of the five most commonly planted trees in New Jersey). In addition, the trees most commonly affected by the disease measured 20 inches or more in diameter. These larger trees are highly valuable to New Jersey landscapes; hazard pruning and replacement costs will be in the hundreds, perhaps thousands, of dollars per tree.

The majority of oak samples positive for BLS were received from counties with the longest history of this disease, although the disease was detected at quite high levels in the western regions of counties that border this area (i.e., Atlantic, Cumberland, and Cape May Counties) (Table 6). BLS of oak has not yet been detected in Passaic, Warren, or Sussex Counties.

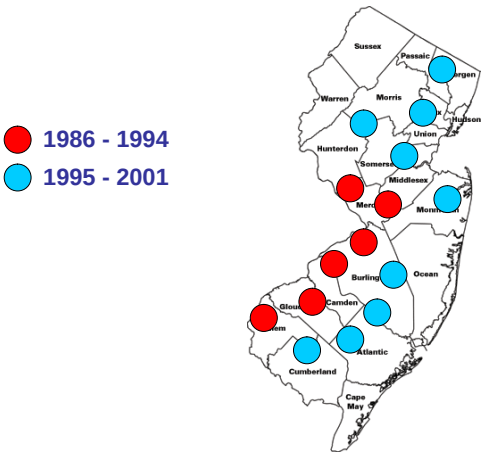

Figure 3. Distribution of BLS from 1986 to 1994 (red dots) and subsequent spread by 2001 (blue dots). (Figure courtesy of A. B. Gould.)

The distribution of BLS of oak within a county is not uniform, and disease incidence in some municipalities is particularly high. For example, BLS affects more than 20% of approximately 1000 oak trees in Moorestown, New Jersey (C. Pfleider, Department of Parks and Shade Trees, personal communication), and we estimate that 30% of oaks in Cranbury, New Jersey are diseased (data not published). These communities are close to natural bodies of water where riparian weeds may serve as a source of bacterial inoculum. Even within a given population of trees, symptom development may occur randomly with no evidence that the disease is moving from one oak tree to the next.

Table 6. Percent of oak samples positive by county for bacterial leaf scorch of oak [50]

County	(%)	County	(%)	County	(%)
Atlantic	58	Gloucester	67	Ocean	9
Bergen	2	Hudson[Z]	---	Passaic	0
Burlington	88	Hunterdon	11	Salem	85
Camden	68	Mercer	63	Somerset	3
Cape May	53	Middlesex	35	Sussex	0
Cumberland	80	Monmouth	34	Union	1
Essex	9	Morris	4	Warren	0

[Z]No samples taken.

TRANSMISSION AND VECTOR ECOLOGY

X. fastidiosa is transmitted by a number of xylem-feeding insects with piercing-sucking mouthparts which include leafhoppers in the subfamily Cicadellidae, and spittlebugs in the family Cercopidae [33; 67]. Both nymphs and adults acquire the pathogen when feeding on succulent branch terminals of infected hosts [46]. Bacteria attach to the cibarial pump and to the lining of the esophagus in an area known as the foregut of the insect [57]. Although *X. fastidiosa* is noncirculative [55], it does divide in the insect, becoming encased in a bacterial glycocalyx that may protect the pathogen and aid in the extraction of nutrients [12].

After acquiring the bacterium, insects are infective within 1 to 2 hours [32; 55]. The number of cells required for transmission may be low. For example, in grapevines affected with Pierce's disease, the number of bacteria required for the glassy winged sharpshooter to transmit the disease to healthy grape was lower than the detectable limit of 100 bacterial cells [31]. As the insect vector begins to feed, pumping action and the flow of fluid through the style dislodge bacterial cells, which are then egested directly into the

xylem. An adult can remain infective indefinitely; nymphs, which shed the foregut during molting, remain infective only until the next molt [57].

Most vector transmission studies of *X. fastidiosa* have been conducted in Florida and California [1; 11; 13; 30; 54; 58; 60; 67; 68]; published studies regarding the identity of possible vector(s) of the bacterium in oak or other shade tree hosts is minimal. In 1994 to 1995, Olszewski [51] captured and identified species of xylem feeding insects in mature oaks in Moorestown, New Jersey (Table 7). Some cicadellids (leafhoppers) are known vectors, but the New Jersey study adds new species as potential vectors. Populations of treehoppers were greatest in early summer, whereas populations of leafhoppers peaked in July and August. Two insects, a *Telamona* species and a *Graphocephala* species, were identified in this study as potential vectors using ELISA on insect extracts.

Table 7. Leafhoppers and treehoppers collected from mature oak trees in Moorestown, NJ, in 1993 and 1994 [51]

Treehoppers	Leafhoppers[Z]
Archasia belfragei	*Alebra albostriella*
Cyrtolobus fenestratus	*Edwardsiana rosae*
Cyrtolobus sp.	*Graphocephala* spp.
Enchinopa binotata	*Oncometopia* spp.
Glossonotus acuminatus	
Ophiderma definita	
O. evelyna	
O. flava	
Ophiderma sp.	
Smilia camelus	
Telamona ampelopsidis	
T. decorata	
O. flavicephala	
O. pubescens	
Telamona sp.	

[Z]14 species were not identified.

During the same time period, Bentz and Sherald [10] and Pooler et al. [52] surveyed oak trees in Washington, D.C. *X. fastidiosa* was detected in the leafhopper species *Aulacizes irrorata*, *Graphocephala coccinea*, *Graphocephala versuta*, *Oncometopia undata*, *Erythroneura* spp., and *Typhlocybia* spp. In Kentucky, Johnson and Freytag [37] extensively trapped treehoppers in pin oak and found that at least six species or genera (including *Enchenopa binotata*, *Ophiderma/Cyrtolobus* spp., *Archasia* spp., *Telamona* spp., *Glossonotus* spp., and *Microcentrus caryae*) could be implicated in disease transmission. The role of any of these insects, indeed there may be more than several species involved, in the epidemiology and spread of BLS, however, is not confirmed, since transmission studies have not been completed.

In Washington DC, Jo-Ann Bentz of the ARS, USNA USDA collected the following insect species and tested them for *X. fastidiosa* using nested PCR: the leafhoppers *A. irrorata*, *G. coccinea*, *G. versuta*, *O. undata*; and the treehoppers *Cyrtolobus fuliginosus* and *Ophiderma definita*. She found that there was much tree-to-tree variation of species and their relative abundance (Tables 8, 9).

Table 8. Populations of insects collected from elm on the National Mall, Washington, D.C.

National Gallery of Art (Madison Avenue)	
Aulacizes irrorata	4
Cyrtolobus fuliginosus	0
Graphocephala coccinea	32
G. versuta	337 (peak in June)
Oncometopia undata	24
Ophiderma definita	0
National Air and Space Museum (Jefferson Avenue)	
Aulacizes irrorata	6
Cyrtolobus fuliginosus	4
Graphocephala coccinea	14
G. versuta	305
Oncometopia undata	30
Ophiderma definita	6

Xylem-feeding insects, particularly leafhoppers, can be polyphagous, feeding on many different hosts within a single season. Specifically, vectors will feed on different hosts during different times in their life cycle in an attempt to derive adequate nutrients for growth and reproduction [14]. Plant hosts suitable for growth, however, may not have nutrients suitable for insect reproduction. This then forces the xylem feeders to use the high-risk strategy of finding new hosts to complete their life cycle. Many of the alternative asymptomatic hosts of *X. fastidiosa* mentioned above serve as a food source for potential leafhopper vectors, and many leafhoppers overwinter as adults on these alternative hosts [59]. Transmission experiments have shown that alternative hosts may be the source of a substantial amount of inoculum that is transmitted to economically important crops by vectors that feed on both types of hosts [3]. The extent to which the relationship between alternative hosts and insect vectors contributes to the disease cycle of BLS in oak and other tree hosts has yet to be elucidated.

Xylophagic insects, such as those that transmit *X. fastidiosa*, usually have a larger body than phloem feeders. Although this may be due to their nutrient poor diet, the larger body size may result in a greater ability of these insects to move between hosts, which for some leafhopper species is a requirement for successful development to adulthood. In addition, there is a level of energy intake that must be met to overcome the negative xylem pressure of the plant. Thus, the larger heads of xylophages make use of a cibarial pump that improves the insect's ability to overcome negative plant pressure and extract fluid from the xylem. Xylem fluid has several characteristics that have forced xylophages to specifically adapt to this feeding strategy. As stated above, the fluid is nutritionally dilute with an unbalanced profile of organic acids where nonessential compounds can predominate. Because of this low nutrient status, adult leafhoppers consume over ten times their body weight per day and have a very high metabolic efficiency. It has become clear from studies in Florida by Russell Mizell and colleagues that to accommodate for differences in nutrition in plants, *X. fastidiosa*-bearing leafhopper species change hosts frequently.

Table 9. Species dispersion indices for populations of insects collected from elm near the National Mall, Washington, D.C.

Species	Dispersion index	Dispersion pattern	Sampling distribution
Aulacizes irrorata	0	regular	Positive binomial
Cyrtolobus fuliginosus	0	---	Positive binomial
Graphocephala coccinea	1.82	clumped	Negative binomial
G. versuta	10.52	clumped	Negative binomial
Oncometopia undata	0.04	regular	Positive binomial

Polyphagy, or feeding on different hosts during the growing season, is related to nutritive value of the host plant [14]. This "host switching" decreases nutritional deficiencies and increases survivorship. By feeding on xylem, the insects avoid toxic secondary compounds and obtain nutrients in an easily assimilated state. The glassy winged sharpshooter (*Homalodisca coagulata*) (GWSS) is a known vector of Pierce's disease of grapevine as well as phony peach disease and plum leaf scald in the southeastern United States.

This leafhopper is an aggressive flyer and is highly polyphagous; to complete development it must often feed on more than one host. Survival of GWSS nymphs is facilitated by a high assimilation efficiency of nutrients, whereas high consumption rates are more important for adult insects [14]. Organic carbon rather than nitrogen may be more limiting for xylem feeders; Anderson and co-workers [6] demonstrate that high feeding rates, a high efficiency of conversion or utilization of organic compounds, and ammonotelism are features that allow the GWSS to subsist on xylem fluid.

In Florida, Russell Mizell has shown that feeding rate and survivorship of the GWSS varies dramatically depending on host (Table 10). The highly variable nutrient status of host plants leads to a high risk lifestyle where insects must change host plants frequently from all nymphal life stages to adulthood. Indeed, the future of leafhopper immatures is dictated by the location at which the female parent decides to lay eggs. Thus, immatures often face the same nutritional profiles as the adult female. Nutrient profiles acceptable for adult feeding results in high mortality of immatures because of poor nutrition. Since nymphs cannot survive well on unbalanced amino acid profiles, they must change habitat to compensate, and since they are not winged, they must jump or walk to a new host. In Florida, Mizell and co-workers have found that GWSS populations progressively changed host plants throughout the season [46]. Because of temporal and spatial qualitative unpredictability of plant species occurrence, this then highlights the risks that adult, and especially immature, vectors face on landscape, patch, and individual plant levels.

In general, GWSS males move from 6:30 PM to dark, and mating takes place at night and early morning. In early evening hours, female receptives wait on plants in the feeding position and likely sonicate the substrate to attract males. Males fly to branches with females and walk in a spiral pattern to the female. Female GWSS face many reproductive tradeoffs when choosing host plants for oviposition (Figure 4). Positioning the entire egg cache in one place may leave all eggs vulnerable to parasitism from mymarid wasps (reported to be close to 100% in the Florida panhandle). Partial egg laying on more than one host results in probable differential mortality due to nutritional variation. In addition, nymphs may have to leave unsuitable host plants in search of a host on which they can successfully develop. On hosts with thicker leaves, eggs deposited deeper within leaf tissue may escape the length of the parasitoid ovipositor, and thus serve as “enemy free space.”

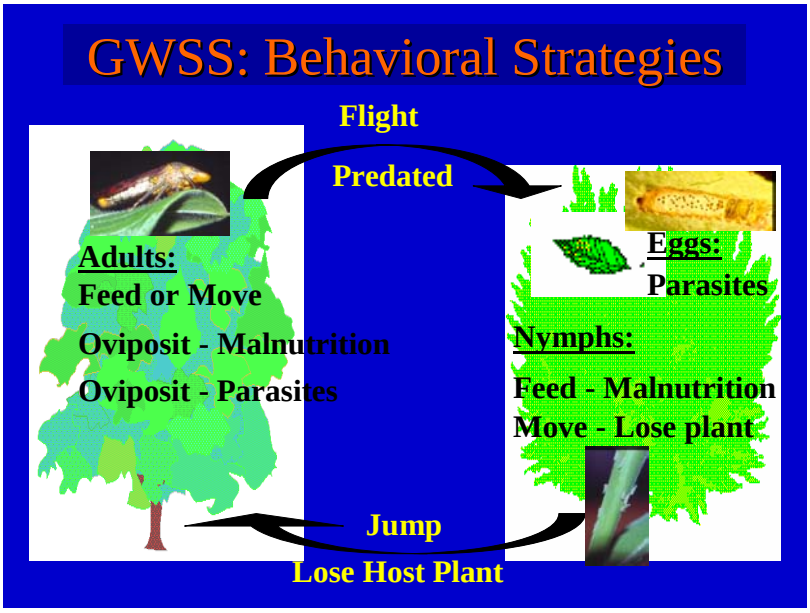

Figure 4. A model of the reproductive and behavioral tradeoffs facing glassy winged sharpshooters during adult and immature stages. These tradeoffs occur because of the low nutritional value of plant xylem, the different requirements of the adults and immatures, and high egg parasitism. (Figure courtesy of R. Mizell.)

Table 10. Feeding rate and survivorship of *Homalodisca coagulata* on two different woody hosts

	Euonymus japonica	*Lagerstroemia indica*
Instar	Feeding rate (ml/day)	
Second instar	0.31	1.62
Fourth instar	0.30	1.57
Adult	1.03	2.62
	Survivorship	
Second instar	5.29	2.87
Fourth instar	4.74	3.39
Adult	1.50	6.25

Hypothetical predicted reproductive outcomes and implied host planting recommendations are outlined in Table 11. Non-host plants for adults can become nominal or temporary hosts for immatures. If these immatures reach adulthood, they can lay eggs on such "suicide" plants and become either heavily parasitized or perish from poor nutrition if they successfully hatch. Alternatively, primary hosts for GWSS such as weeds, shrubs, and trees can provide adequate nutrition for all life stages, but most eggs will become parasitized. Enemy free hosts support all life stages and the eggs are not parasitized. In all cases, mortality from predation can occur. To better understand the development of BLS in economically important shade tree hosts, these specific feeding and egg laying activities of insect vectors must be made clear.

Table 11. A model of predicted reproductive outcomes for *Homalodisca coagulata* and implied host planting recommendations by host plant category

Type	Adult	Egg	Nymph	Parasite	Predicted outcome
Non-host	N	Y/N	Y/N	Y/N	plant
Primary host	Y	Y	Y	Y	remove
Adult only host	Y	Y/N	Y/N	Y/N	trap crop
Nymph only host	N	Y/N	Y	Y/N	---
Enemy free host	Y	Y	Y	N	remove
Suicide host	Y	Y	N	Y/N	trap crop

IMPACT OF MOISTURE STRESS ON DISEASE DEVELOPMENT

There is much anecdotal evidence that leaf scorch symptoms associated with BLS are more severe if plants are stressed, particularly by drought (Figure 5). To test this hypothesis, McElrone from the University of Maryland examined the relationship between disease development and moisture stress in Virginia creeper (*Parthenocissus quinquefolia*). This host of *X. fastidiosa* occurs naturally throughout the eastern half of the United States and is an important component of natural forest communities as well as in ornamental plantings. When infected by *X. fastidiosa*, the host displays a prominent, irregular leaf scorch preceded by a chlorotic halo [35].

In 1999 and 2000, McElrone examined symptom progression and growth response, water relations, gas exchange, and leaf chlorophyll content of infected (I) and noninfected (NI) Virginia creeper subject to high (HW) and low (LW) moisture levels. In each year, a high percentage of inoculated plants expressed

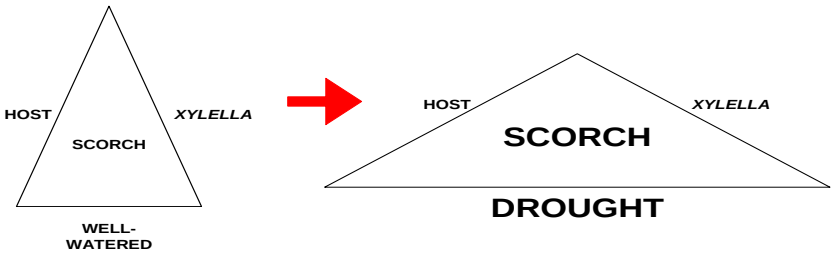

Figure 5. The component that most often drives the disease process is environmental conditions conductive to disease development. (Figure courtesy of A. McElrone.)

typical bacterial symptoms that progressed along the stem from the inoculation point. In both years, symptoms progressed further and were more severe at corresponding leaf positions in LW-I plants compared to HW-I plants (Figure 6). Total leaf area, shoot length, and number of nodes on the longest shoot was additively reduced by both LW and I treatments.

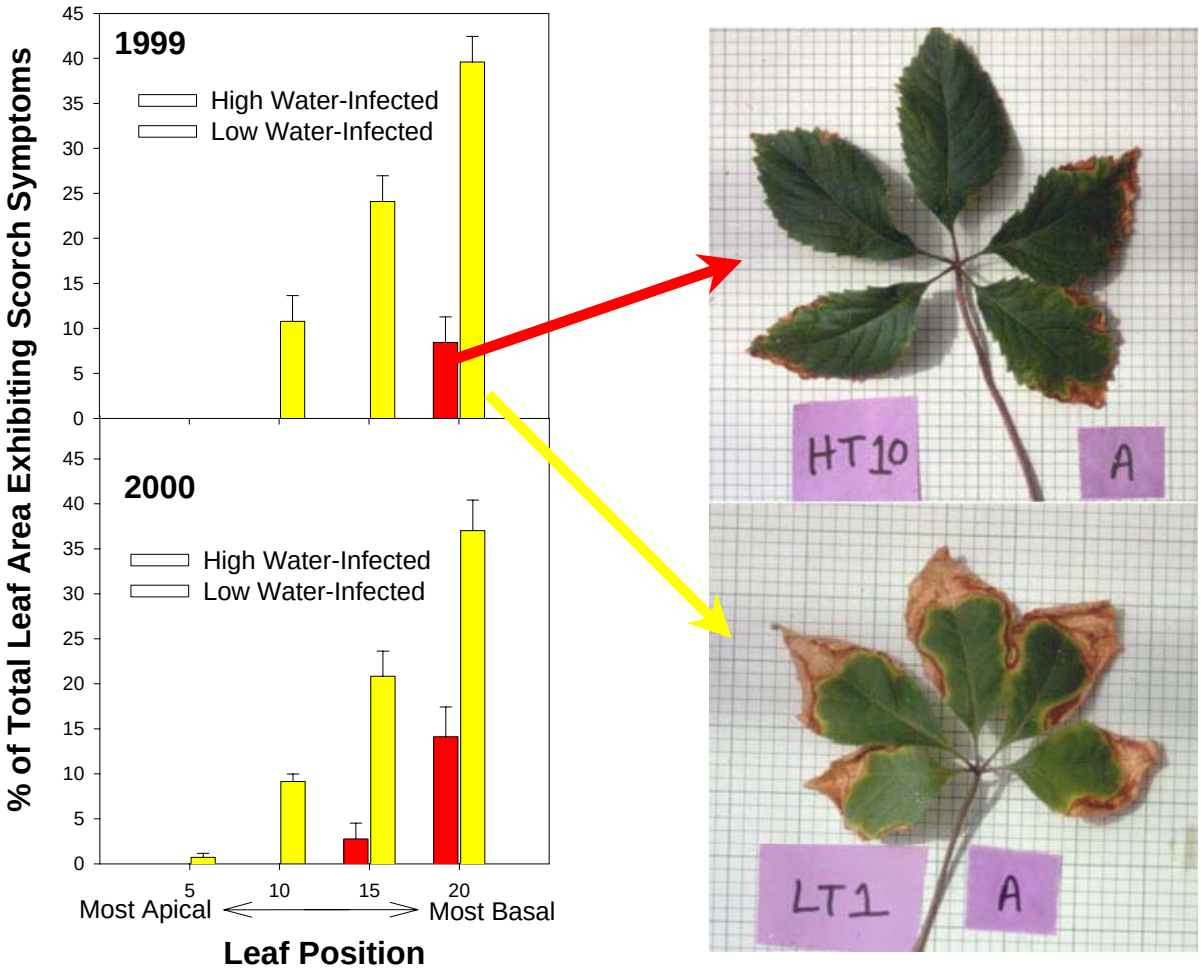

Figure 6. Impact of water treatment on symptom expression in Virginia creeper inoculated with *Xylella fastidiosa*. (Adapted from [45], photographs courtesy of APS Press.)

Leaf water potential, net photosynthesis, and stomatal conductance were reduced in the LW treatment at all leaf positions, whereas these parameters were reduced in I treatments at basally located leaves only. Whole shoot hydraulic conductance and xylem vessel lengths were reduced by both LW and I treatments, while LW increased percent embolised vessels and reduced vessel diameter. These results imply that the major effect of infection occurs due to reduced hydraulic conductance caused by clogging of the vessels and not by increased cavitation and embolism of xylem elements. However, embolism may be involved in disease progression and the development of symptoms earlier in the growing season or in tissues other than the stem (i.e. petioles) and should be assessed in future studies.

The reduced hydraulic conductance caused by *X. fastidiosa* acts additively with the water limitation imposed by drought. Throughout most of the growing season Virginia creeper responded to LW and I by reducing stomatal conductance at moderate levels of water stress, which reduced leaf photosynthetic rates. Toward the end of the growing season, increased intercellular CO_2 concentrations, reduced leaf nitrogen content, and reduced photosynthetic rates at high CO_2 concentrations, where stomatal limitation was eliminated, indicated that severe stress induced by I and LW eventually lead to decreases in photosynthesis associated with accelerated leaf senescence. The combined reductions in photosynthesis caused by LW and I reduce the plant's ability to outgrow and defend against *X. fastidiosa*.

This study is the first to verify the hypothesis that bacterial leaf scorch symptoms are enhanced during drought stress. Maintaining plant vigor with regular watering can be used to sustain plants infected by *X. fastidiosa*, particularly during periods of water stress.

DISEASE DETECTION

X. fastidiosa is detected in host plants and insect vectors using light microscopy, electron microscopy, isolation on selective media, serologically through immunofluorescent staining and enzyme-linked immunosorbent assay (ELISA), standard PCR techniques, and immunomagnetic separation followed by PCR, which has been particularly useful in vector studies. Although detection using ELISA remains the most rapid and cost-effective method to detect *X. fastidiosa* in symptomatic tissues, genomic techniques, which are more sensitive, are necessary for detecting low populations of bacterial cells in vectors or in infected, asymptomatic hosts. Pathotypes of *X. fastidiosa* are identified by pathogenicity tests.

Due to its fastidious nutritional requirements, *X. fastidiosa* is difficult to culture on standard media, and several selective media have been developed for this purpose [16-19]. Ramone Jordan (USDA) compared and described different techniques used to detect the bacterium in insect extracts and plant tissues (Table 12). *X. fastidiosa* is best isolated from symptomatic tissues and is identified by its characteristically slow growth and small, opalescent colonies. Culture assays are based on the growth of *X. fastidiosa* from plant samples plated onto one of these selective media. The number of live bacteria in the sample can be determined from the number of colonies that grow on the plate. Culture assays are fairly sensitive (down to thousands of bacterial cells per gram) and are highly reliable if the cultured bacteria are further confirmed as *X. fastidiosa* by other means. Disadvantages of this method, however, are that they take more than a week to complete, other bacteria and fungi compete with the pathogen and obscure results, and certain plants (e.g., black walnut and coffeeberry) contain substances that inhibit growth of *X. fastidiosa* on culture medium.

Table 12. Summary of detection methods for *Xylella fastidiosa*

Assay	Number of samples comfortably accommodated	Sensitivity[Y] (number of bacterial cells)	Cost	Labor
Culture	10-100s	1000	Low	Low
ELISA	100-1000s	100,000	Med	Med
PCR	100s	100	High	Med
IC-PCR[Z]	100s	<100	High	High

[Y]Lowest approximate number of bacterial cells that the assay can detect.
[Z]Immunocapture-PCR

Other serological and genomic techniques are faster but are unable to distinguish live bacterial cells. Of these, ELISA is relatively inexpensive, and there are commercial test kits available that are easy to use. Although the sensitivity of this technique is very low (the lower detection limit is 100,000 bacterial cells per sample), it is very useful if symptomatic tissue is used.

PCR is the most sensitive (100 cells per sample) of detection techniques, can be used for frozen or preserved samples, and can be used to distinguish some strains of *X. fastidiosa*. PCR is generally not quantitative, however, is expensive and labor-intensive, and is not widely available in diagnostic laboratories. In addition, some naturally occurring chemicals can inhibit the PCR reaction, resulting in negative tests. In nested PCR, the PCR process is done twice, resulting in a more refined 500 bp product.

Of the detection techniques developed thus far, however, PCR preceded by an immunocapture step removes normal inhibitors of the PCR reaction and improves detection limits. Small magnetic beads that are coated with antibody to *X. fastidiosa* from rabbits are added to a pool of insect or plant extracts (Table 13). The beads capture bacterial cells, and when washed free of inhibitors are used in the nested PCR reaction.

Table 13. Detection of *Xylella fastidiosa* from insects using immunocapture-PCR

Insect species	Samples tested	Positive samples
Graphocephala coccinea	77	19
G. versuta	453	45
Erythroneura spp.	222	3
Typhlocybia spp.	43	2
Membracidae	127	7
12 other species	156	0

DISEASE MANAGEMENT

Management of the many diseases caused by *X. fastidiosa* has encompassed various strategies, including reducing host stress [20; 63], use of resistant varieties [43; 48], and removal of infected hosts [63], insect vectors [2; 53], and alternative hosts [59]. Jim Sherald suggests that there is little evidence that therapeutic pruning, trenching, and early removal of infected trees are useful disease management techniques. In addition, as in peach, infected hosts may remain asymptomatic for long periods prior to symptom expression [21].

The use of antibiotics such as oxytetracycline to control *X. fastidiosa* has been attempted on hosts such as grapevines in a soil drench [34], and as trunk injections in several hosts [38]. Relief from symptom development is temporary, however, and antibiotics must be reapplied each year to be effective. Kostka et al. [38] reported that injection was most effective when less than 20% of the canopy was symptomatic for leaf scorch prior to treatment.

Currently, a single antibiotic compound (Mycoject®, J. J. Mauget Company) is labeled for leaf scorch of elm and red oak. Part of Bruce Fraedrich's responsibilities at the Bartlett Tree Research Laboratories is to support the company's commercial operations with experimental trials of potential BLS management products. He started experiments in 1988 to treat oaks with the antibiotic oxytetracycline as a trunk injection using Mauget capsules (Figure 7). In 1987, 10 trees in a stand of red oak in Wilmington, Delaware, were evaluated for leaf scorch (% of crown with scorch symptoms). The following June, oxytetracycline was applied at 100 mg active ingredient per inch (dbh) and evaluated for symptoms three months later. During the year of treatment (OTC 88,89 and OTC 88), symptom expression was reduced approximately 55 to 80%. Leaf scorch remained low in trees injected again the following June (OTC 88,89), but returned to 1987 levels if left untreated (OTC 88). Injecting twice the label rate was of no added benefit (OTC 89 (2X)). Fraedrich surmises that the antibiotic reduces bacterial populations in vessels so that plugging is not as severe or results in a delay in symptom development until much later into the season.

Further studies in the mid-1990s with ferric ammonium citrate (a common treatment for iron chlorosis) and Aliette (a systemic pesticide with some bactericide properties) did not yield any meaningful results. In 2000-2001, Fraedrich examined oxytetracycline in a powder formulation (labeled for palm trees), a tree growth regulator call Profile, and Hydrostat, which is a bactericide that has a wide variety of uses in personal healthcare products. In late summer of 2001, good disease suppression was evident with oxytetracycline, and there was enough activity with Profile and Hydrostat to encourage further studies with the products.

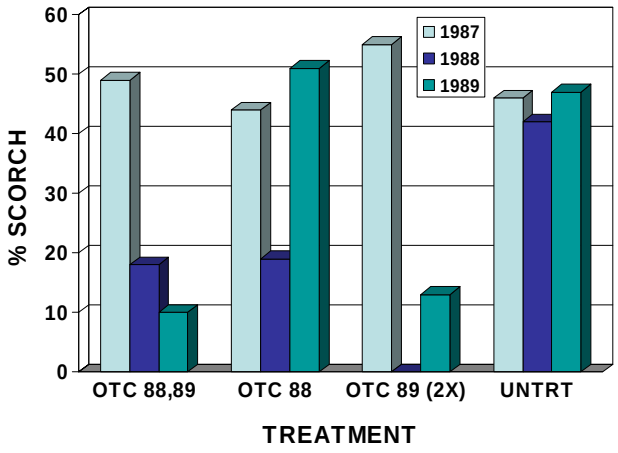

Figure 7. Impact of oxytetracycline treatment on symptom expression in red oak. (Adapted from B. Fraedrich presentation.

Fraedrich also assessed the impact of fertilization on symptom development in diseased trees. In April 1998, application of a 28-9-9 slow release fertilizer at the rate of 6 lb N/1000 sq. ft. surface area (the upper range of standard recommendation for tree fertilization) reduced symptom expression approximately 20%, but results were not statistically different from control trees.

Management of the diseases caused by *X. fastidiosa* through vector eradication has been attempted, but even with Pierce's disease of grapevine, where disease vectors are known, results are inconclusive [2; 53]. Adlerz et al. [2] theorize, however, that insecticidal sprays may reduce the spread of Pierce's disease; in cases where insect populations rise dramatically, sprays may be necessary to prevent this disease from spreading to adjacent areas [59]. Preliminary studies conducted by Fraedrich indicate that use of the systemic insecticide Merit® (imidacloprid) to reduce vector populations in sycamore may have some efficacy for disease management. This compound is effective for homopteran control up to one year when applied to trees as a basal drench or soil injection. Since vector control using insecticide sprays has little value, the use of Merit on diseased

trees may possibly reduce the movement of infective insects from tree to tree. In 2002, Fraedrich plans to study the impact of Merit on disease incidence in a long-term sycamore trial.

At this point, what strategies may be helpful for management of BLS in amenity trees? The most useful disease management tool may be to alleviate water stress by using mulch and irrigating trees during times of drought. Although therapeutic pruning is not recommended at this time for disease control, removal of dead limbs is necessary to prevent invasion of secondary diseases and pests and to prevent hazard situations. Management of other pests (e.g., anthracnose, Dutch elm disease, obscure scale, borer, and cankers) and abiotic stresses (e.g., moisture stress and iron chlorosis in pin oaks) may prolong the life of infected trees. Fertilizing trees is recommended only if they are nitrogen deficient. Planting recommendations for municipalities, community foresters, and landscape architects are to use a mix of different tree species and avoid planting highly susceptible trees in known areas of disease. Currently, the only bactericide option available has mere bacteriostatic ability; further studies are needed to determine whether continual antibiotic injection is a sustainable control method and whether these compounds may be phytotoxic to trees treated for long periods [38].

FURTHER RESEARCH AND OUTLOOK

Although the cause of BLS is known, questions concerning the prevalence and distribution of BLS in New Jersey and other states remain. In addition, the identity and life habits of specific insect vectors are not known, and reliable epidemiological and insect sampling techniques for determining the extent of insect infection cycles and inter-host movement are not established. Detection techniques used by Olszewski, Pooler, and others are useful but primitive, and more disciplined sampling techniques must be developed to obtain useful data in tree communities. Pooler et al. [52] reported that in some species, as few as 1% of insects may be infected with *X. fastidiosa*, so collection of large numbers of insects is critical. As more information becomes available about treehopper and leafhopper behavior in oak trees, placement of insect traps in the canopy to maximize collection will be useful, especially in cases where populations of a given species are low. Proof that insects collected from trees are, indeed, vectors must also be obtained. Although Olszewski [51], Pooler et al. [52], Johnson and Freytag [37] and Bentz [10] have implicated several xylem-feeding insects as potential vectors of BLS, transmission studies for these and other insects to prove their roles as vectors must be completed.

Other issues of concern include the identity and role of alternative, “potential-reservoir” hosts on disease development and the identification of potential disease management practices. To date, BLS is troublesome in New Jersey in certain species of oak, although the host range of *X. fastidiosa* encompasses several other amenity trees that commonly occur, and are occasionally infected in New Jersey landscapes (e.g., maple, sweet gum, and mulberry). It is clear that the transmission and spread of BLS is complicated, and considerable knowledge must be acquired for detection, prediction, and management of BLS in communities, parks, and golf courses.

Another key to understanding the BLS problem is differentiation, geographical distribution, and host pathogenicity (including cross pathogenicity) between bacterial strains. Understanding what strain affects what host is very important for making management decisions such as tree removal and replanting.

From a practical standpoint, the value of therapeutic pruning, especially on newly infected trees, should be assessed. This type of study will be facilitated by development of more rapid, on-site diagnostic techniques for this disease. In addition, possible spread of bacteria from tree to tree on pruning tools or by grafting should be evaluated. Further work with insecticides and promising bactericides should continue to help prevent spread or alleviate chronic symptoms. The susceptibility of some tree species such as the elm hybrids is unknown and needs to be evaluated, and sources of resistant germplasm in oak and sycamore populations should be identified and screened.

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